MDDC - 991

UNITED STATES ATOMIC ENERGY COMMISSION

THE EFFECTS OF IRRADIATION ON THE BLOOD AND BLOOD FORMING TISSUES

by

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DTIC QUALITY INSPECTED 2

Date of Manuscript: January 11, 1943
Date Declassified: April 6, 1946

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Technical Information Branch, Oak Ridge, Tennessee
AEC, Oak Ridge, Tenn., 2-23-49--850-A3656

Printed in U.S.A.
~~PRICE 10 CENTS~~

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By S. T. Cantril, L. Jacobson and J. J. Nickson

ABSTRACT

A review is given of the effects of external whole body and local irradiation in man and experimental animals. Internal irradiation by radioactive substances in man and experimental animals is considered. An abstract of pertinent findings is given in the summary and conclusions.

THE ORIGIN AND DIFFERENTIATION OF BLOOD FORMING TISSUE

All investigators are in agreement that blood formation occurs embryologically in an identical manner in all mammals. It consists of a condensation in the yolk sac of the embryonic connective tissue, the mesenchyme, into blood islands, the central cells of which "round" up into basophilic cells which give rise to the primitive erythroblasts. These first basophilic cells have been called large lymphocytes by Maximow,[60] hemocytoblasts by Bloom,[10] Maximow,[59] and by many other names. The blood cells produced in this embryonic site are largely so-called primitive red corpuscles which acquire hemoglobin, remain nucleated, and carry oxygen. As the embryo grows, primitive cells migrate to other sites and they, as well as local mesenchymal cells in these sites give rise to the definitive generation of red corpuscles, granulocytes, megakaryocytes, lymphocytes and other blood cells. The production of the cellular elements of the blood in places other than the yolk sac proceeds in a definite pattern (see Bloom[7,11,59]), probably in the following order: liver, spleen, lymph nodes, and bone marrow. No division of function into production of myeloid and lymphatic cells occurs in any of these sites until late in embryonic life.

In the human at the time of birth, with the exception of minute foci in the liver which soon disappear, erythropoiesis and myelopoiesis have been taken over entirely by the bone marrow, and the production of lymphocytes and monocytes by the lymphatic system—lymph nodes and spleen. Only under pathological conditions such as pernicious anemia, leukemias, etc., does erythropoiesis or myelopoiesis ever occur in the adult liver, spleen, or lymph nodes (extramedullary hemopoiesis). In some mammals, however, notably the mouse, and to some extent the rat and rabbit, the spleen is active in the formation of practically all blood elements even in the adult.

An important fact to remember is that the bone marrow normally produces the erythrocytes, the granulocytes, and the megakaryocyte series, and the lymphatic system produces the lymphocytes. The site of production of monocytes is somewhat controversial, but experimental evidence compiled by Bloom[8,18,62] and others seems to indicate that their production is also a function of the lymphatic system.

Under normal conditions the body tends to maintain the cellular elements of the peripheral blood in fairly constant numbers. The mechanisms accounting for these phenomena are generally unknown except perhaps in the case of the erythrocyte where the degree of peripheral oxygenation regulates the rate of production, release from spleen, etc.

Under normal conditions homoplastic hemopoiesis accounts for the supply of mature erythrocytes

and granulocytes, that is, very few hemocytoblasts are differentiating into these elements. The constant supply for the peripheral blood is maintained by division of the young forms (erythroblasts and myelocytes, respectively) and subsequent maturation. Under pathological conditions, on the other hand, heteroplastic hemopoiesis occurs. This occurs when extreme demands are being made upon the bone marrow. The hemocytoblast then comes to the rescue with the production of new erythroblasts and myelocytes.

The granular cell series develops from the primitive stem cell (hemocytoblast) within the bone marrow and has a number of readily recognizable maturation stages, namely promyelocyte, myelocyte, metamyelocyte, and lastly the lobulated neutrophil, eosinophil, or basophil. All the primitive forms, including the metamyelocyte, are capable of mitotic proliferation. The neutrophil which is of the most importance in a discussion of this group of cells maintains itself normally at relatively constant numbers in the peripheral blood. The total average leukocyte count per cu mm of blood is given by Wintrobe[99] and others as 7000, with a minimum of 5000 and a maximum of 10,000. Of this number, the neutrophils comprise 57% to 67% (or 4300, with a minimum of 3150 and maximum of 6200). The alterations in the absolute and relative numbers of leukocytes may proceed with tremendous rapidity and often denote the nature of a noxious agent. The number and degree of immaturity of the neutrophils are a good index of bone marrow stimulation. The neutrophils are actively motile,[12,99] phagocytic,[30,57] contain proteolytic enzymes[5,74,89] and consume oxygen.[85] The normal life span of neutrophils is not definitely known. From various observations it is usually considered by most authors to be in the vicinity of a week or less.[12,99] Under abnormal conditions as in inflammation, the life of the neutrophil may be exceedingly short. The degenerating or dead neutrophils are disposed of in numbers of ways. In inflammation one observes phagocytosis and digestion of these cells by local phagocytes; normally one finds degenerating forms in the urine, feces, saliva, and menstrual secretion. The reticulo-endothelial system as a whole and in particular that part of it in the liver and spleen plays a part in their disposal by the action of local macrophages.

The lymphocyte originates in the lymphatic system from the primitive reticular and other lymphocytic cells. One recognizes three types of lymphocytes in lymph nodes and the spleen, namely, the large, medium, and small. The large and medium lymphocytes are considered by Maximow[60] to be morphologically identical with the hemocytoblast, or stem cell, which gives rise to the granular, monocyte, and erythrocyte series, and others. It is likely that all forms of the lymphocyte are capable of proliferation.

The average, minimum, and maximum numbers[99] are given as 2100, 1500, and 3000, respectively, and comprise between 25% and 35% of the total leukocyte count. What has been stated previously regarding alterations in absolute and relative numbers of leukocytes applies as well to the lymphocytes. The lymphocyte is actively motile,[12,80] is capable of transition into monocytic forms which are actively phagocytic,[8,18,45] contain enzymes,[5,15] and consume oxygen and sugar.[85]

The normal life span of a lymphocyte is, as in the case of the granular series, not definitely known. There is indirect evidence to indicate a relatively shorter life than the neutrophil. Studies made on thoracic duct lymph[59,102] indicate that larger numbers of lymphocytes are present in the lymph of the thoracic duct than can be accounted for in the peripheral blood and, in addition, many of the lymphocytes encountered here are in process of mitosis.

The origin of the monocyte is a most controversial subject. It is believed by Sabin[84] and her co-workers[20,100] and by Forkner[25] that the monocyte is derived from a specific monoblast and has a definite maturation series. Bloom,[8,18,62] on the other hand, has good evidence to indicate that the monocyte is merely a transition from a lymphocyte. In general, however, most hematologists agree that the monocyte is not normally produced in the bone marrow but rather in the lymph nodes and spleen with the notable exception of Isaacs[39] who believes they are normally formed in the bone marrow. These cells are actively phagocytic and play specific roles in certain infections, but in general little else is known regarding their other properties. Nothing definite is known regarding their life span.

The red corpuscle normally originates exclusively in the bone marrow in the adult human. As in the embryonic liver, the erythroblast is derived from a primitive stem cell and early acquires characteristics which mark its destiny. A well-recognized series of maturation forms can be seen and identified in the bone marrow from the erythroblast stage to the mature red corpuscle. Multiplication appears possible during the entire normoblastic stages. The reticulated erythrocyte represents that stage in the development immediately after the normoblast has lost its nucleus. This stage has been shown by various researches to be the most sensitive to destruction by various agents. At some stages during the normoblastic development hemoglobin is acquired, but its function in oxygen and carbon dioxide exchange is utilized in the adult under normal conditions only when the non-nucleated form is released into the peripheral circulation. It seems likely that oxygen and carbon dioxide have a role in maintaining a normal acid-base relationship (through the shift of the chloride ions from cell to plasma) and in the maintainence of the viscosity of the blood. That the mature erythrocyte consumes oxygen seems to be accepted.[44,79] Normal adult males and females maintain an average of 5 to 6 million and 4 to 5 million erythrocytes per cubic millimeter and 14 to 18 g and 12 to 16 of hemoglobin per hundred cubic centimeters of blood, respectively. The hemoglobin iron is derived from Fe ingested through the gastro-intestinal tract. Normally there is no hemoglobin iron loss as the hemoglobin iron which diffuses into the plasma upon destruction of the worn out erythrocyte is stored and redistributed to newly formed cells. The erythrocyte level is apparently maintained by stimuli to the bone marrow as a result of increased or decreased oxygen tension in the blood itself. In pernicious anemia, where there is a maturation arrest in the megaloblast stage, an average of 7 days is required after liver therapy is instituted before the appearance of the reticulocyte. The life span of a mature erythrocyte has been estimated by several workers in several ways. Whipple[96,97] et al studying bile pigment output in dogs estimated an average life span of 124 days. Ashby[3] measured the length of time transfused blood persisted in the recipient by tests for agglutinogens and observed an average of 83 days; Debbers,[19] using the same technique, found 54 to 74 1/2 days. The manner of final destruction and disposal of the red corpuscle is not definitely known but, in general, investigators agree that the reticulo-endothelial system as a whole, and in particular that part of it in the spleen, serves in the phagocytosis of the degenerating and abnormal forms of the erythrocyte.

The platelets of the peripheral blood have been a subject of great controversy. This controversy has been centered largely on their origin and function. It is generally accepted today that the platelets are derived from the megakaryocytes found in the bone marrow and perhaps the lung.[38] The megakaryocyte itself is thought to originate from the same stem cell which gives rise to the other blood elements

Normally about 250,000 platelets are present per cubic millimeter in the peripheral blood. It is generally accepted that the platelets play a part in the coagulation of blood and in clot retraction.

The life span of the platelet is not known, but is thought to be quite brief, probably 3 or 4 days.

THE LIFE CYCLE OF THE LYMPHOCYTE AFTER IRRADIATION

Effect on Lymph Follicles

Irradiation has a very definite and marked effect on the production of lymphocytes. The majority of papers are in essential agreement as to the presence and nature of this effect (Henicke,[32] Murphy and Nakahara,[68,69] Hilbert and Linzer,[36] Lacassagne and Lavedan,[50] Aubertin and Beujord,[4] Ducuing, Milesky, and Marques,[22] Henshaw,[34,35] and Tsuzuki[93]).
Following acute exposure to whole body irradiation in animals, the effects are in general:

Three hours after irradiation a sudden breakup of the lymphocytic nuclei into chromatin masses is observed. This permits the epithelioid cells of the germinal centers to be seen easily.

Four hours after irradiation there is a migration of polymorphonuclear cells into the irradiated nodes. These begin to phagocytize the debris.

Eight hours after irradiation the nuclear destruction is at its height.

Twenty-four hours after irradiation, the cells of the germinal centers in the lymph nodes, thymus, Pyers patches, and cortical layer of the thymus are all necrobiotic. Many neutrophils and macrophages are seen and phagocytosis is at its height. This process continues for the next 48 hours.

Seventy-two hours after irradiation the polymorphonuclear neutrophils have disappeared, leaving the lymphoid stroma almost devoid of cells. Repair begins, at the earliest, in 4 days and varies in onset and time needed with the exposure. Recovery, once begun, is quite rapid following single doses of the order of 200 to 300 r.

Autopsies done on dogs exposed to doses of whole-body radiation[22] so massive that they produced a rapid and marked lymphopenia, showed wide spread destruction throughout all the lymphoid organs. In the spleen there were patches of necrosis; the normal architecture had disappeared with complete disappearance of the Malpighian corpuscles. The sinuses were filled with macrophages containing blood pigment. The normal architecture of the lymph nodes was destroyed; the germinal follicles had disappeared. There was a fairly general increase in the volume of the lymph nodes due to (a) dilatation of the sinuses which were filled with blood, and (b) hypertrophy of reticular tissue.

Henshaw and Nettleship[34] showed destructive nuclear changes in the cells of the reticulo-endothelial tissue after 50 r whole-body irradiation in mice. The initial changes were observed one hour after irradiation was given. In addition to the nuclear changes, a shift to an acidophilic staining cytoplasm was observed. After 24 to 48 hours, numerous giant cells were observed within the organs of the reticulo-endothelial system. Repair was rapid, and was completed within 96 hours.

In vitro experiments with cultures of lymph nodes, spleen, and thymus have been carried out by Lacassagne and Gricouroff.[5] Cultures were made from lymph tissue obtained from rabbits 1 to 8 days old. Irradiation of cultures was done with x rays with the following factors: 80 kvp, 12 ma, filter 1 mm Al, t.s.d. 27 cm, time 30 to 60 minutes, doses 2000 to 4000 r. In each instance there was destruction of the lymphocytes. The site of origin of the culture did not affect the reaction measurably, indicating little specific organ difference. They also irradiated a 12-day rabbit with 300 r to the body using the same factors, then obtained culture material immediately after irradiation. Destruction, arrest of maturation, and failure of division of the lymphatic cells was again observed.

Osgood and Bracher[75] have irradiated lymphocytes obtained with bone marrow. X rays were used with the following factors: 200 kvp, 7 ma, 1 mm Cu and 1 mm Al, t.s.d. 70 cm, 13.8 r/minute, 400 r dose. They found a drop in the lymphocyte count of the culture to 50 per cent of the normal in about 36 hours. There was a further drop to about 10 per cent of normal (compared with controls) by the 4th day. The level of lymphocytes varied from 10 to 20 per cent for the remaining 3 days at which time the experiment was concluded.

The question of varying sensitivity of lymphocytes from different origins has been studied, Regaud and Cremieu[77] showed that there is some difference in sensitivity in the thymic lymphocytes. Using x ray in "moderate doses" they showed that the cortical cells were more rapidly and completely destroyed than were the medullary cells. They were also able to show that the cortical cells were more completely destroyed when they were in the periphery of the cortex. Their explanation for this differential sensitivity of the thymic lymphocyte was in the age of the cells—the younger, more sensitive cells being in the cortex, the older cells being in the medulla. This was further supported by the observation of a transient decrease in the number of the medullary cells about one week after irradiation, after the cortex had begun to be repopulated with lymphocytes. It was thought that the interference with lymphocyte production in the cortex manifested itself in one week by the failure of appearance of these cells in the medulla.

Lacassagne and Gricouroff[48] concluded that there was little to point to a difference in lymphocyte sensitivity due to site of origin in various organs. Tsuzuki[93] found a definite difference in the sensitivity of the lymphocytes of various origins in the rabbit. The lymphocytes of the portal lymph

glands showed destruction after 75 r. Those of the mesenteric lymph glands were not affected until 150 r had been given. Those of the lymph follicles of the spleen were unchanged until a dose of 225 r had been given. He emphasized that, although there seems to be a difference in threshold of destructive effects, the destruction of the lymphocytes of the spleen is more complete than in the organs affected earlier. There also seemed to be a greater ability to regenerate, i.e., the lymph nodes were capable of more rapid and complete repair than were the splenic follicles.

Henicke[33] in 1913 was able to demonstrate nuclear destruction in lymphocytes in the exposed spleen or intestine of the rabbit or guinea pig after 0.25 r (gamma). The exposure was 20 mg radium for 5 seconds. The radium was contained in a varnished ebonite container which was encased in a rubber sheath. An exposure of ten minutes to 20 mg radium, same factors, plus 3 mm lead was necessary to produce the same changes (approximately 25 r). One hour exposure (168 r) through the intact abdominal wall was necessary to produce nuclear destruction in the spleen and lymph glands of the intestine.

Murphy and Nakahara,[68] using x rays for body radiation in mice (spark gap 7/8 inch, 25 ma, t.s.d. 8 inches, time 20 minutes, no filter) found changes of a different nature than heretofore described in the germinal centers.

Twenty-four hours after irradiation a slight increase in the number of mitotic figures in the lymph follicles was observed.

Forty-eight hours after irradiation a marked increase in mitosis was observed.

Ninety-six hours after irradiation the number of mitotic figures had begun to decrease, and seven days after irradiation little increase in mitosis was noted.

They believed this response was primarily of a "stimulative" nature, as opposed to the "destructive" features evident with higher doses or with more penetrating radiation. The penetrating radiation. The penetrating quality of the rays used is small. Murphy and Nakahara[69] showed that 50% of the radiation was absorbed in the first quarter centimeter of tissue using x rays from a tube with the following factors: spark gap 1/2 inch, 11 ma, t.s.d. 6 inches. X rays as used above would have 50% absorption within the first 1/2 cm. Thus it is difficult to see how x rays of this type can exert any direct effect on the lymphocyte-forming organs, as many of them lie deeper than 1/2 cm from the skin. It may be that some product of the interaction of the skin indirectly produces these changes. It may be, however, that these observations are a manifestation of repair to damage not recognized before 24 hours had elapsed, when their first observations were made.

Effects on Circulating Lymphocyte

The evidence to date seems to point to an absence of effect on the circulating cell. Lacassagne and Gricouroff[51] have irradiated in vitro specimens of blood of the rabbit with doses varying from 200 to 3500 r (2H to 35H) (150 kv 7 ma, t.s.d. 30 cm, filter 1 mm Al). With doses of less than 2000 r, no deviation from the normal motility was observed. Above 2000 r the majority of lymphocytes had lost motility. In another experiment, a rabbit was given 2000 r with the same factors, then bled, and the blood cultured. No deviation from the control cultures was seen. The animal died on the 3rd day post-irradiation, with a white blood count of 430 cells per cu mm, of which only 10 per cent were lymphocytes. They concluded that there was a definite, though feeble sensitivity of lymphocytic elements in the blood, and that the threshold for this effect seemed to be about 2000 r.

Shouse and others,[88] in a similar experiment, were unable to find any effect on the circulating lymphocytes with doses as high as 3500 r.

Effect of Irradiation on the Number of Circulating Lymphocytes

Apparently only very small doses are necessary to produce some change in the peripheral blood picture. The decrease in number is to some extent a function of the exposure of body irradiation given. The duration of the effect is, however, directly influenced by the total amount of radiation given.

Russ, Chambers, Scott, and Mottram[82] using soft x rays, found a 50 per cent reduction of lymphocytes after 1/5-second exposure of body irradiation in rats. The drop did not exceed 50 to 60 per cent with increase of exposure up to 30 minutes. The length of recovery time was prolonged after administration of the higher doses. With 12-second exposures the lymphocyte counts recovered in 24 hours; 1-minute exposures required one week for complete recovery; 30-minute exposures required 3 weeks for recovery; 60-minute exposures were lethal. Leich[56] has questioned the validity of these figures, especially for the smaller exposure times. Russ,[81] however, maintains that the figures are reproducible.

The picture seen with therapeutic doses of irradiation in experimental animals has been described by numerous investigators,(Ducuing,[22] Henshaw,[35] Lacassagne and Lavedan,[50] Piney and Mayneord,[76] Aubertin and Beujord,[4] Russ, Chambers, Scott, and Mottram,[82] etc.). They are all in essential agreement. In all animals yet investigated the findings are essentially the same, with doses of the order of 200 to 300 r of whole-body radiation. The lymphocyte count is found to be effected at the latest 1/2 hour after the beginning of irradiation. The initial count may be halved in 4 to 6 hours. Within the first few days it commonly drops to 0 to 300 cells per cu mm. The time of recovery is dependent upon the exposure, but for single doses of the order mentioned, the count begins to rise in about 3 weeks. Once recovery is initiated, it proceeds quite rapidly. Following repeated irradiation, the count is depressed for longer periods of time. The individual exposures need not be larger than 15 to 25 r/day (Ducuing, Marques, and Milesky[21,22]).

The following are some examples of the effect of varying exposures:

Rabbit— Piney and Mayneord[76] using x rays (90 kvp, 3 ma, t.s.d. 25 cm, no filter) found the following results: One animal received 5090 r in 4 1/2 hours. (Figure 1). The initial lymphocyte count was 1900. In 4 hours the count was 2000; in 8 hours, 400. For the next 62 hours, the lymphocyte count did not rise above 1000, and for the next 32 days did not rise above this level.

Another animal was given 7920 r divided in 7 equal daily doses. The lymphocyte count initially was 4700. On the 2nd day it had dropped to 2000; on the 3rd day to 200; thereafter the lymphocyte count was below 500 until death, which occurred on the 24th day after irradiation was begun.

An exposure of 190 r daily at the rate of 19 r per minute was given to another rabbit (Figure 2). Initial lymphocyte count was 4000; 2nd day, 1900; and 4th day, 1000. Thereafter no lymphocyte count was above 800 cells per cu mm. Exposures were given for 21 days or a total of 3960 r. Counts were taken for 56 days postirradiation, at which time the lymphocyte count was still around 800 cells per cu mm.

Exposure of 1 minute a day for 23 days, a dose of 22 r per day to a total of 502 r, produced the following changes: Initial lymphocyte count 3400; 2nd day, 3400; 3rd day, 3600; 8th day, 2900; 12th day, 3300; 18th day, 2500; 24th day, 2000. In no case did the lymphocyte count drop below 1200 and this figure was reached 8 days after irradiation ceased.

Another animal was given 490 r in a single exposure (Figure 3). The lymphocyte count the day before irradiation was 3400; 2nd day, 2800; 3rd day, 1400. The lowest count was reached on the 8th day when the count was 600. By the 14th day the count had risen to 1500. Thereafter the count ranged between 3000 to 1000. Counts were taken for 116 days after exposure to x rays.

The animal receiving 7920 r in 7 days died on the 23rd day. The animal receiving 5090 r in a single exposure was followed for a period of 32 days, but the authors do not give information concerning this animal after the 32-day period. We are surprised to learn that the animal lived as long as 32 days with this exposure. The exposure of 200 r daily for 21 days and observations carried over a period of 56 days is also a dose which we would have expected to be lethal before 56 days. In comparing these figures with lethal doses in other animals (dogs as cited by Ducuing, or guinea pigs and mice as used by Henshaw), there seems to be a wide discrepancy. Either there is a large specie variation in sensitivity, or the physical factors of dosage measurements are not comparable.

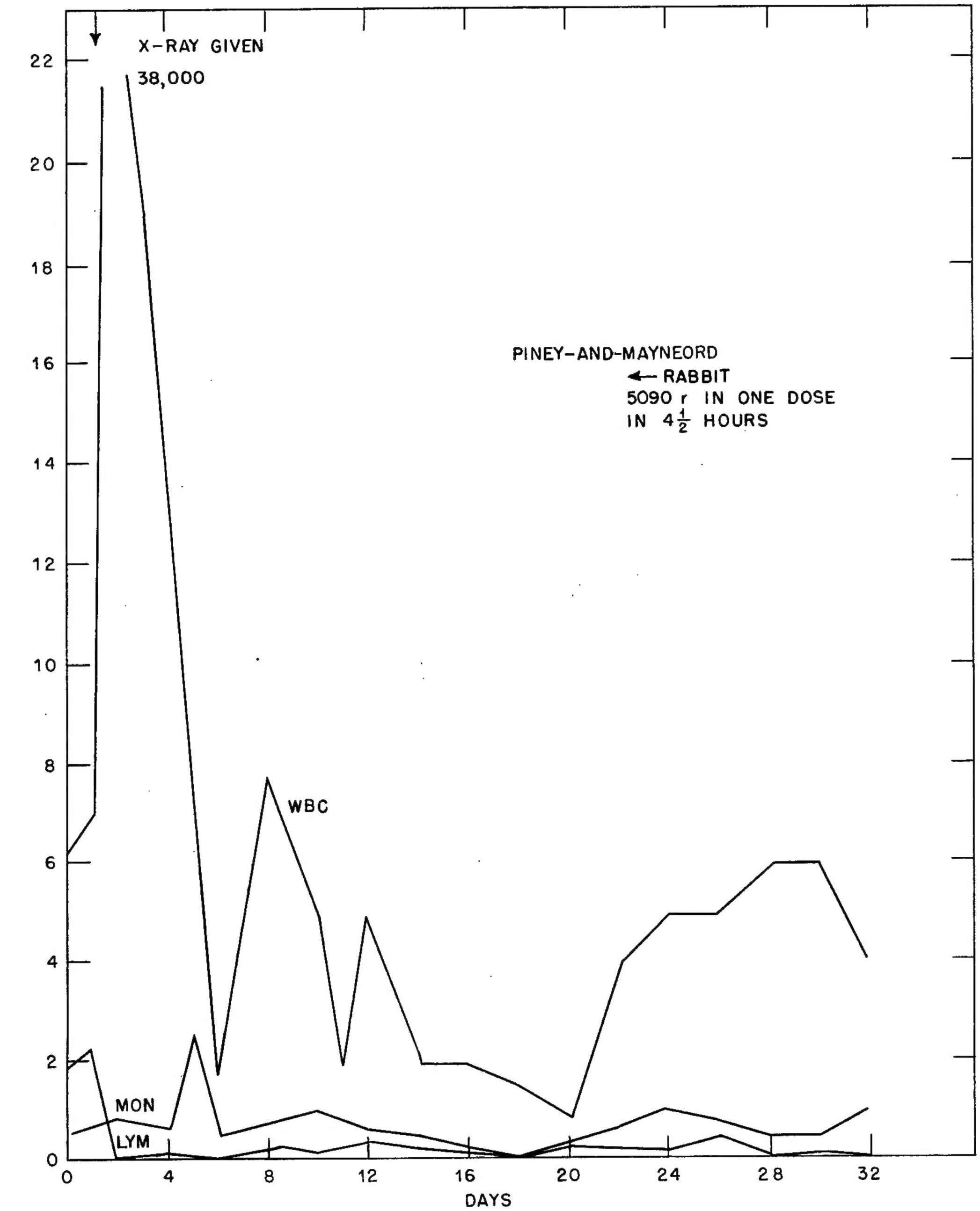
X-RAY GIVEN
38,000
PINEY-AND-MAYNEORD
RABBIT
5090 r IN ONE DOSE
IN 4½ HOURS
WBC
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Figure 1.

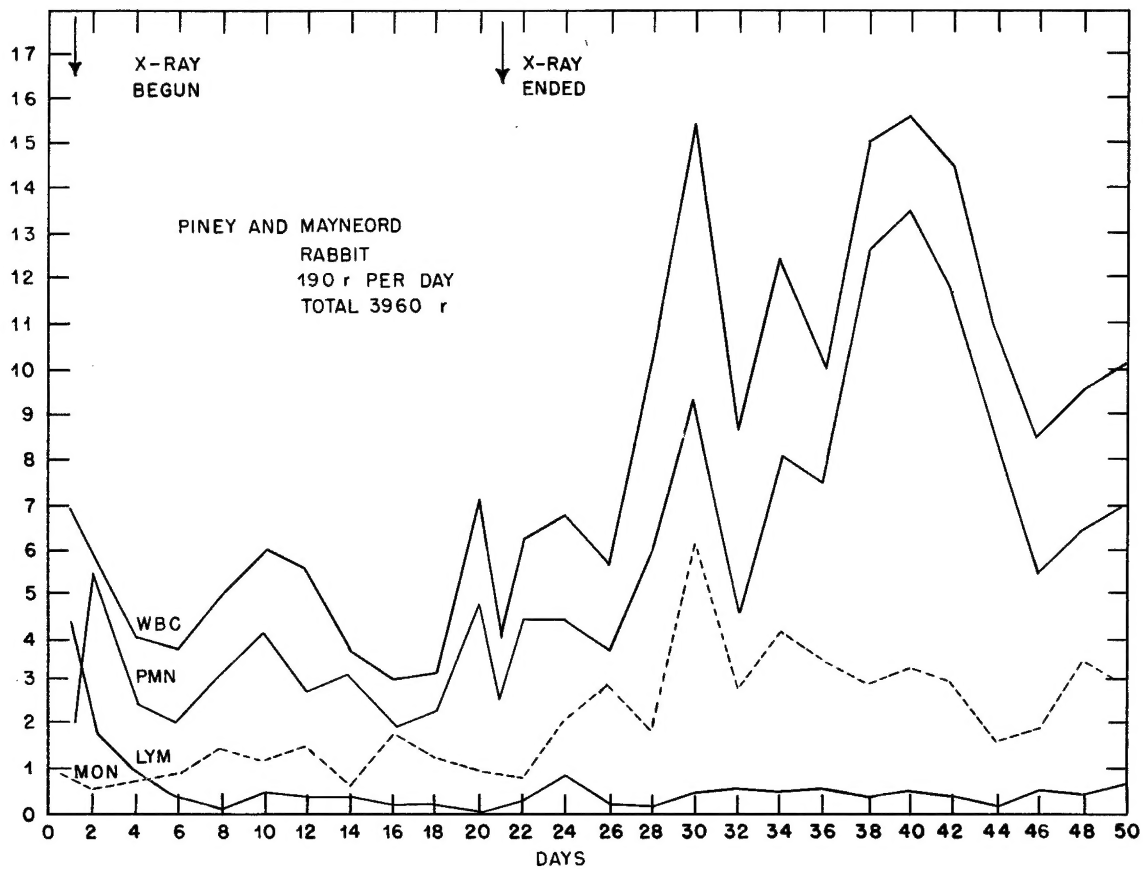

Figure 2.

Ducuing et al[22] have studied blood effects after whole-body radiation in dogs. (180 kv, 0.5 mm Cu, 150 cm). The results are recorded in accompanying graphs for doses of 1425 r given in 35 days (Figure 4); 775 r given over 82 days (Figure 5); 100 r given daily for 12 days (Figure 6); and 500 r given as a single exposure (Figure 7). In all these examples except the 775 r given over a period of 82 days, a rapid and persistent drop in the lymphocytic level is seen, which does not rise appreciably; the drop is to 100 to 700 cells per cu mm. In the exposure of 775 r carried over 82 days, the lymphocytes fell to around 700 cells within 6 weeks after irradiation was begun, remaining at this level throughout the remainder of the exposure and beginning to rise 2 weeks after irradiation was terminated, reaching a level of 1600 lymphocytes (pré-irradiation level 2800) 6 weeks after cessation of irradiation. Five months after termination of irradiation the lymphocytic level was still 1600, with variations below this level in the interim.

Shouse, Warren, and Whipple[88] gave dogs an estimated 3 SED (200 kv, 35 ma, 0.5 mm Cu, 1 mm Al, 60 cm, 2 fields each 900 cm^2, abdomen shielded with 1/8-inch lead). The lymphocytes dropped to 50 to 0 cells per cu mm by the 5th day and remained at that level until death, which occurred on the 9th day.

Lavedan[53] followed the blood counts of patients being treated for carcinoma of the cervix. The irradiation in this case was not total-body irradiation, but local irradiation of the pelvis and lower abdomen with large fields. Factors: 180 kvp, filter 2.5 mm Zn, t.s.d. 50 to 75 cm, 100 r in 60 minutes, pelvic fields 375 to 450 cm^2, the number of fields varying with the individual case.

After a single pelvic exposure with a dose of 100 r, there was, in general, a leukopenia which reached its lowest level within 2 to 3 hours with a slight lymphopenia. The white blood count began

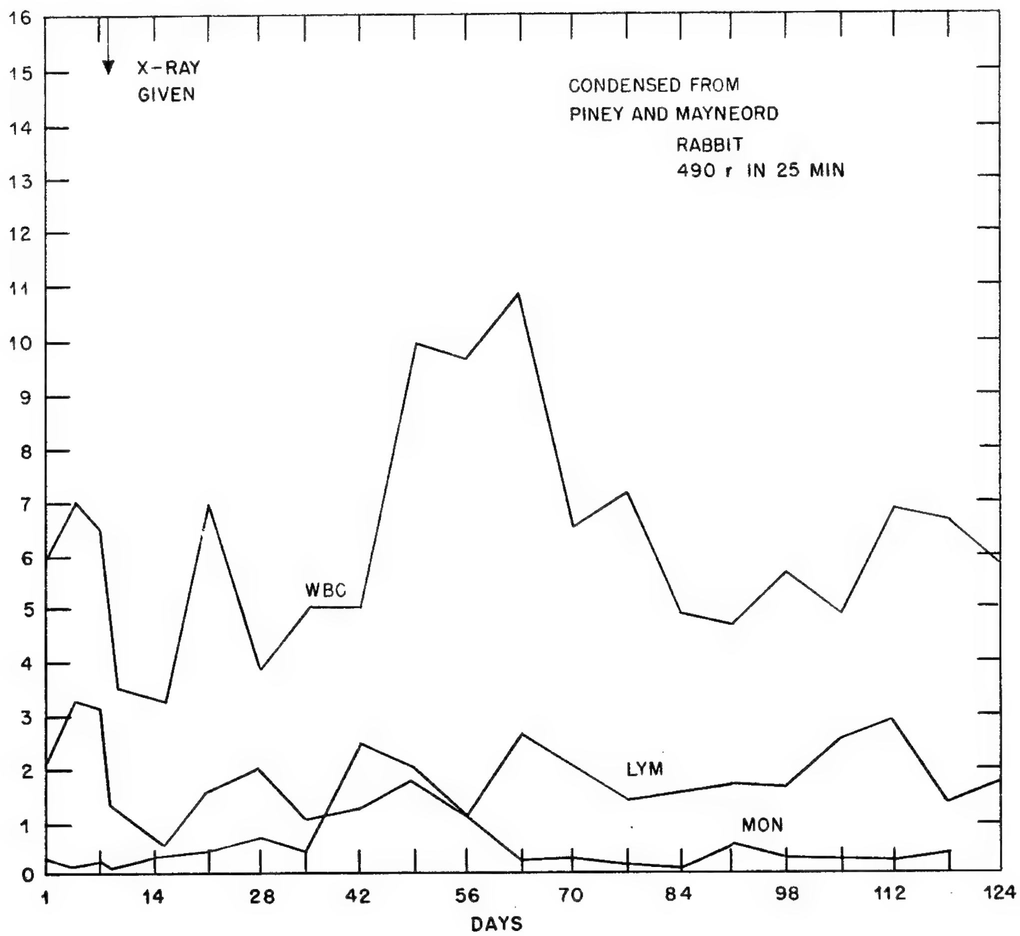

Figure 3.

to rise between 3 to 5 hours and reached or somewhat exceeded normal limits within 24 hours (leukocytosis with slight neutrophilia). In the course of the complete pelvic irradiation (10,000 to 12,000 r given in 25 days) there was found to be a progressive leukopenia (loss of 50 per cent) with lymphopenia which returned to normal limits within 4 to 5 weeks.

Minot and Spurling[63] reported on the blood findings in 12 patients irradiated with x rays (200 kvp filter .05 mm Cu, t.s.d. 75 cm, average field size 940 cm^2). The total dose was from 338 to 1950 ma.m. In all instances treatment was completed in 4 days. Lymphocytes dropped to an average of 1200 within the first 3 days. The rise in lymphocytes began on the 6th day and took from 4 to 6 weeks to return to normal. The length of the recovery period was a function of the magnitude of the dose.

Goodfellow[29] followed a large number of patients who had been treated with radium both as implants and surface applicators. With doses of the order of 3000 mghrs. or above there was constant finding of lymphopenia (drop averaging 50 per cent) which was at its height by the 3rd to 7th day, depending upon the rate of dose. There was also a very constant, though transient, reversal of the lymphocyte-monocyte ratio which seldom persisted. The cases were not followed long enough to give information concerning the rate of recovery. The size of the areas irradiated varied widely, as did the sites of irradiation. It is of interest that the effect on the blood count was more a function of the dose than of the site of irradiation. It is also possible here that the amount of scattered radiation to the whole body with the very high doses (100,000 mghrs.) may account for some of the changes observed.

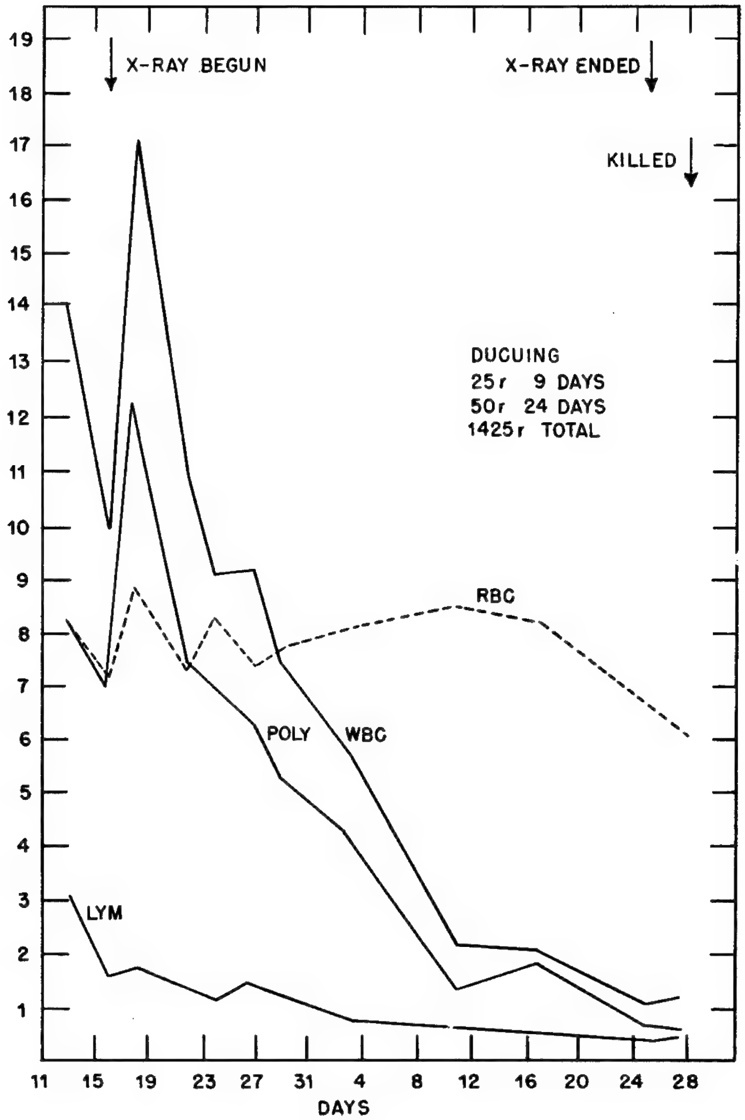

Figure 4.

Lavedan[54] also reported on the changes in the blood of professional workers. He believed that the most characteristic change following exposure was a relative and absolute lymphocytosis, with a tendency for the lymphocyte-monocyte ratio to become inverted. Goodfellow[28] also felt that this increase in lymphocytes was the earliest and most reliable sign of overexposure in professional workers. The next most common response was a lymphopenia associated with a monocytosis.

Stimulating Effect of Radiation on Lymphocytes

Taylor, Witherbee, and Thomas[90] using x ray of the following factors: Spark gap 7/8-inch, ma 25, t.s.d. 8 inches, time 20 minutes, no filter, produced changes of a somewhat different nature in the circulating lymphocytes than those described above. Using rabbits, the initial lymphopenia was transient, of only a few hours duration, and was followed by a rise to as high as 200 per cent of the initial pre-irradiation level. This lymphocytosis persisted for as long as 42 days. They further

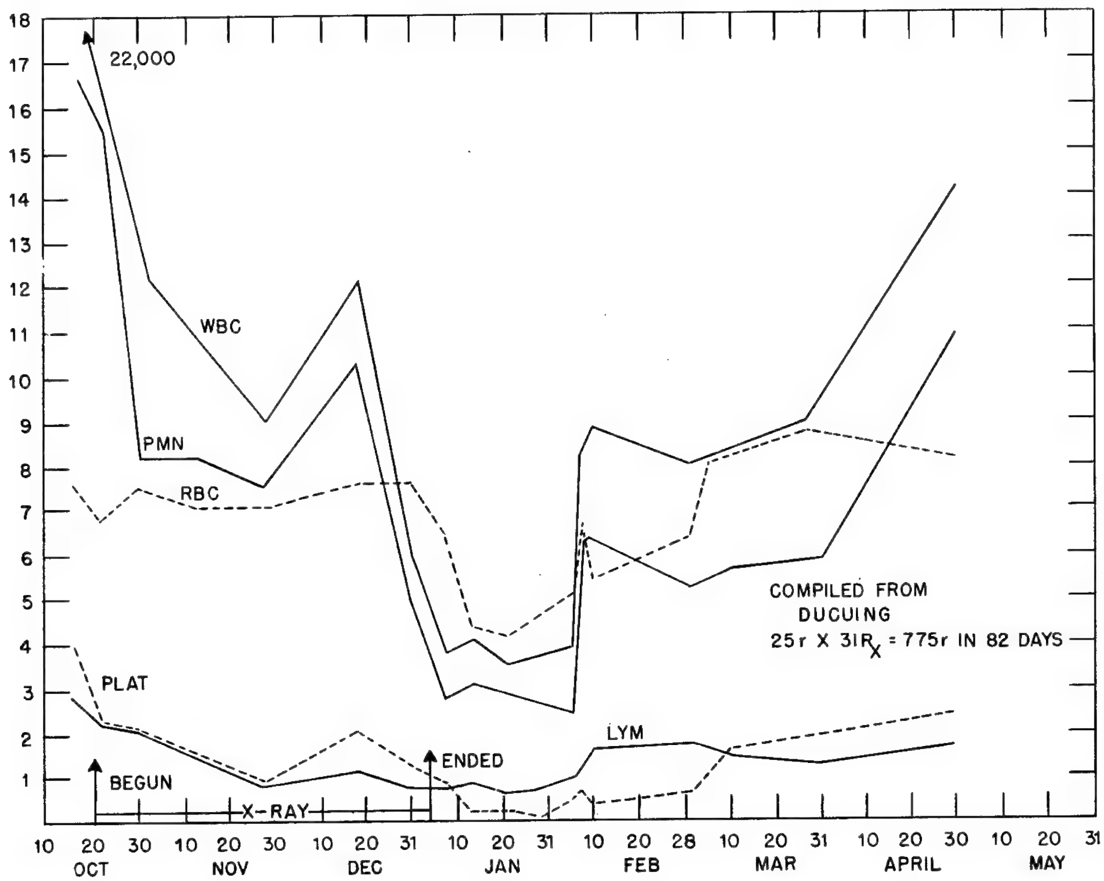

Figure 5.

noted that if the time of irradiation was lengthened to 27 minutes, using a 3 mm Al filter, the lymphocytosis did not appear.

Murphy and Nakahara[68,69] and others[61,91] using x ray with 0.5-inch spark gap, 11 ma, t.s.d. 6 inches, exposure 1 minute, found an elevation of the lymphocytic count up to 150 per cent in mice. The increase, when present, persisted on an average of two weeks.

Russ and others[81,82,83] have confirmed the foregoing findings in most essentials. They find a more decided initial lymphopenia than that described. By giving repeated 12-second exposures (t.s.d. 30 cm, Coolidge tube, 4-inch spark gap, 12-sec exposure = 1/200 "rad") about every 3rd week, these investigators were able to build up the lymphocyte count in two rats to 112,000 and 117,000, respectively, in a period of 28 weeks. The initial levels were 11,000 and 25,000, respectively. They were also able to produce lymphocytosis with daily exposures of 12 sec each over a period of 8 days. The rise in lymphocyte count was from 34,000 to 66,000.

The explanation for this effect of very soft irradiation is obscure. One possible explanation is found in the presence of a similar response following exposure to heat[73a] and after injection of oleic acid or olive oil.[72,73] It is possible that the x rays or heat act as a nonspecific stimulus which results in the formation of break-down products in the skin which act as a stimulus to the germinal centers. Inasmuch as it has been calculated that these rays are 90 per cent absorbed in the outer 1/4 cm of tissue, there can be no question of direct action on the lymphocytes in the lymph follicles within the deeper structures. No adequate explanation has as yet been put forward for this effect of very soft irradiation on the lymphoid tissue and upon the number of circulating lymphocytes.

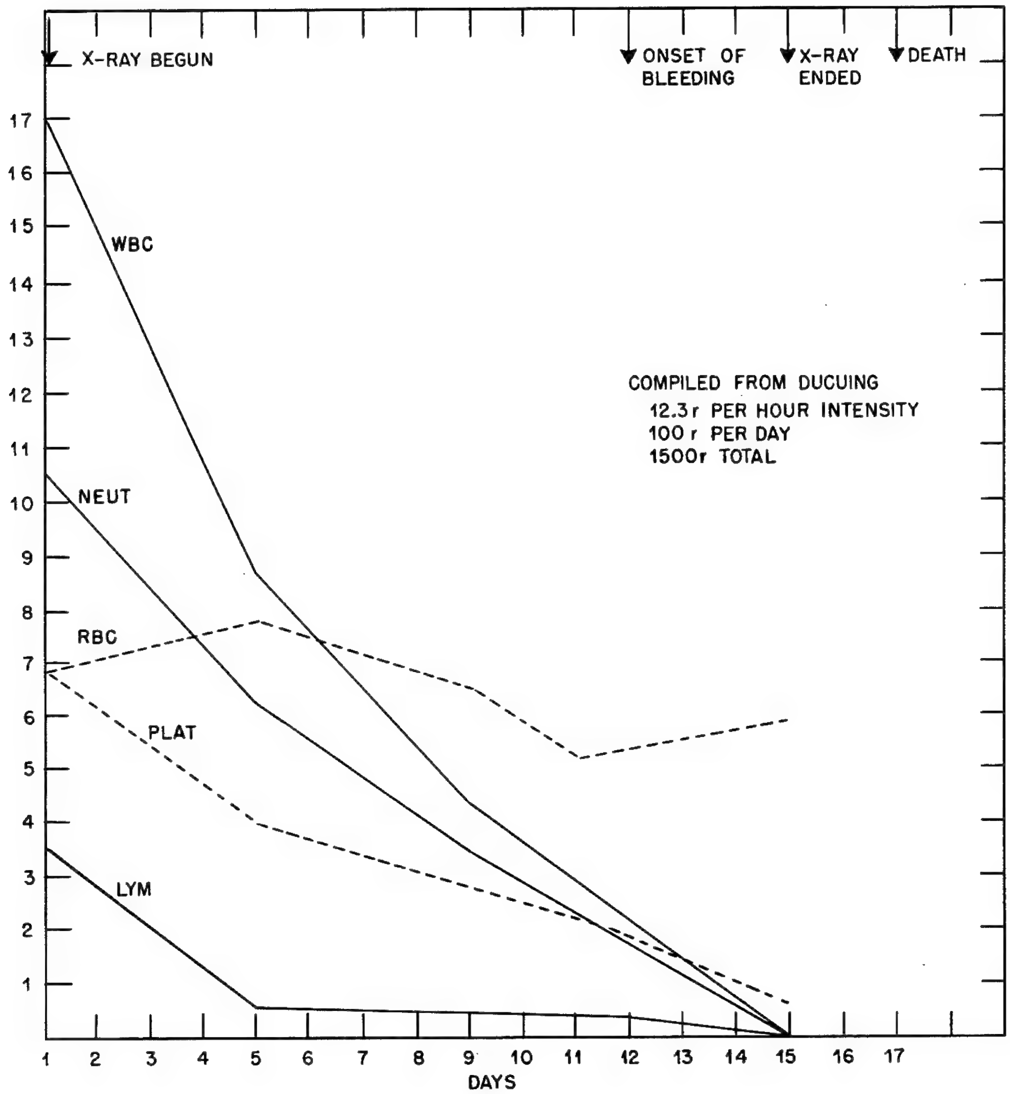

Figure 6.

Monocytes

The site of origin and the parent cell of the monocytes is as yet undecided. Hence, little can be said concerning the sensitivity of their parent cells. In experimental animals, no characteristic variation has been demonstrated in the numbers of circulating monocytes. Piney and Mayneord (Figure 1) were able to produce in a rabbit an inversion of the lymphocyte-monocyte ratio after 5090 r in 4 1/2 hours. However, this was done more by reducing the lymphocyte level to very low levels, (0 to 500 per cu mm). In another instance (Figure 2), 3960 r whole-body irradiation (190 r per day for 21 days) was given a rabbit. The monocyte level varied between 800 to 1800 cells per cu mm (initial level 800) during the period of observation. However, after irradiation had ceased, the number of monocytes rose to as high as 6200 (total white blood cells 15,000) by the 9th day. This peak lasted for a few days only; during the next twenty days their number varied between 4000 and 1800. Their level never returned to the pre-irradiation levels. 7920 r total-body irradiation in the rabbit, given in 8 equal doses of 880 r per day (1-hour exposure) produced a definite monocytosis with reversal of the lymphocyte-monocyte ratio on the 4th day after irradiation was begun and persisted as long as the animal was followed (27 days). The initial monocyte level was 500 cells per

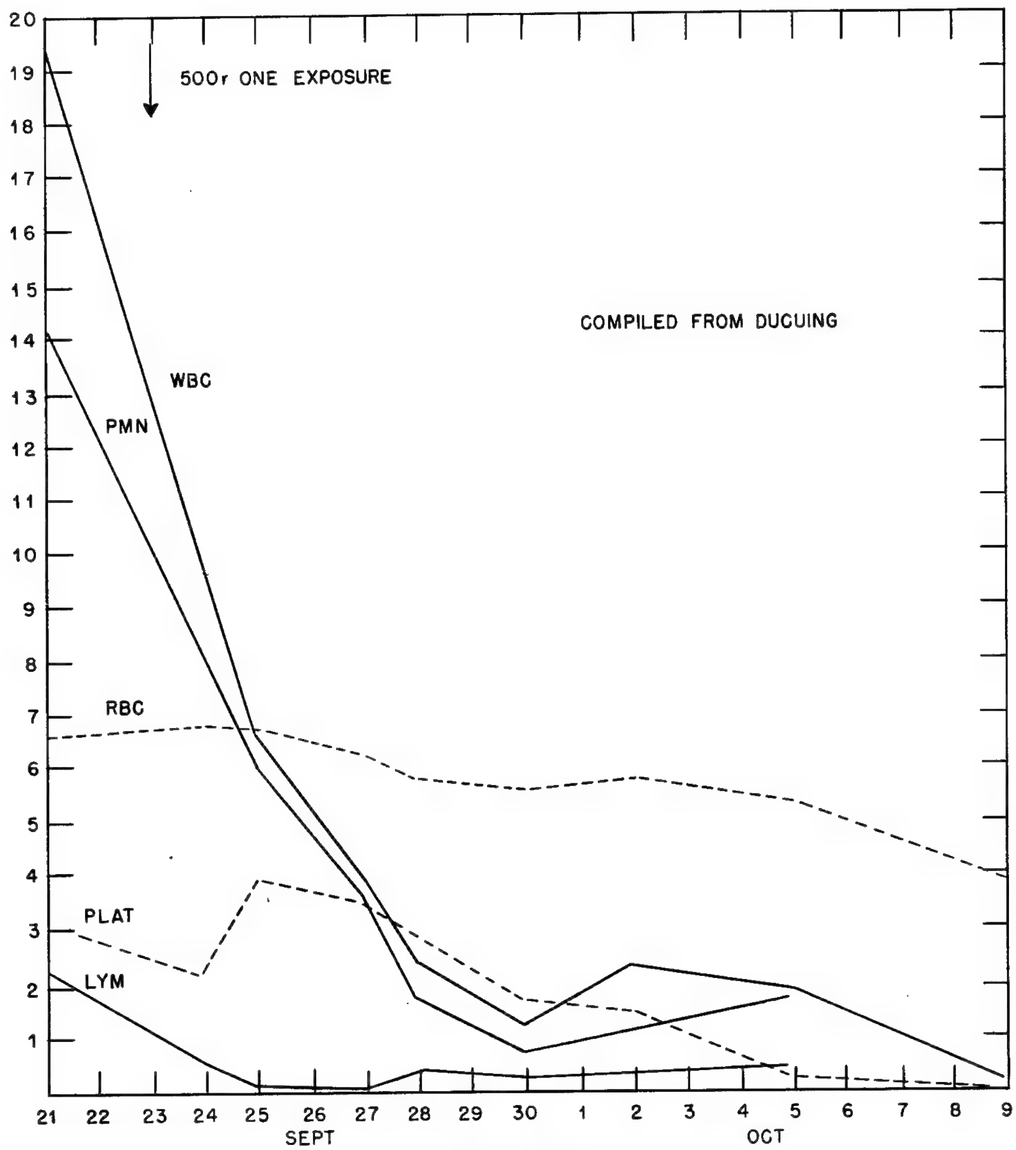

Figure 7.

cu mm. By the 4th day, they had dropped 200, and subsequently began to increase irregularly, reaching a peak of 2000 by the 20th day.

In humans exposed to whole-body irradiation, Goodfellow[28] feels that one of the more serious prognostic signs after exposure to radiation is reversal of the lymphocyte-monocyte ratio, with an absolute monocytosis. Goodfellow[29] in a large series of patients has shown that elevation or depression of the monocyte count may occur after irradiation with gamma rays. In 55 cases, 18 showed little change in the monocyte values, 20 showed a rise that at some time as least doubled the initial monocyte value, and 18 showed at least one count that halved the initial level. No distribution could be seen correlating effect with dose level. The doses ranged from 1440 to 110,000 mghrs.

LIFE CYCLE OF THE NEUTROPHIL AFTER IRRADIATION

The initial effect of radiation upon the neutrophils would seem to be an acceleration of their maturation in the bone marrow with a consequent liberation into the blood stream of abundant newly formed polymorphonuclear leukocytes. This mechanism also serves to explain the leukocytosis (largely a neutrophilia) which follows the initial leukopenia (largely a lymphopenia) and is seen in the circulating blood some 3 to 12 hours after the first exposure to radiation. Whether or not the acceleration of maturation is of a sufficient degree to be evident by neutrophilia depends upon the initial exposure. If this is one which is overwhelming, the destructive effects on the bone marrow may be such that even a transient acceleration of maturation of neutrophils is overshadowed by the neutropenia resulting from destruction of the leukopoietic cells of the marrow. It would seem then that, within limits, the reaction of the leukopoietic function of the marrow has a quantitative relation to the dose of irradiation.

The acceleration of maturation of the neutrophils as an initial response to irradiation is described by Brauner and Gottlieb[6] in following what they term the "myelogram," or picture of the bone marrow, at varying times after an initial dose of radiation in the human. 28 r whole-body irradiation in man (200 kv, 3 meters, 0.25 mm Cu plus 1 mm Al, 4 r/m) produced a shift in the ratio of nonsegmented to segmented polymorphoneuclear leukocytes of the following order:

	Before irradiation	8 min	8 hr	24 hr	72 hr
Nonsegmented	6.8	4.4	5.6	0.8	3.2
Segmented	28.4	36.0	24.0	62.8	38.4

The same effect is recorded by the myelogram of the sternal marrow when a relatively small dose of localized radiation is given to the pelvis: 220 r given to ischial field 20 by 20 cm (200 kv, 25 ma, 50 cm, 0.5 mm Cu plus 1 mm Al) on 2 successive days produced the following shift in the maturity of neutrophilic cells in sternal marrow:

	Before irradiation	48 hours
Neutrophilic myelocytes	10.0	1.6
Polymorphonuclear neutrophils		
Nonsegmented	18.8	2.4
Segmented	18.5	62.0

That the acceleration in maturation of the granular neutrophils is not maintained with prolongation of the dose is demonstrated by the following study. The irradiation of a single pelvic field with a dose of 203 r (200 kv, 25 ma, 50 cm, 15 by 15 cm^2, 1mm Cu plus 1 mm Al) produced an increase to 56.5 per cent in the segmented polymorphonuclear neutrophils of the sternal marrow within 72 hours. When this irradiation was then continued to a total dose of 2200 r, the myelogram returned to a level of 17.2 per cent segmented polymorphonuclear, which was the pre-irradiation or normal level. It would thus seem that acceleratory effects were counterbalanced by inhibitory or destructive effects on the irradiated marrow by the same dose repeated up to 2200 r.

In both of these latter examples the acceleration of maturation seen in the sternal marrow resulted from irradiation of tissue other than the sternal marrow itself.

Early evidence of the initial neutrophilic leukocytosis following irradiation was demonstrated by Aubertin and Beujord[4] in 1905. Whole-body irradiation of the mouse, guinea pig, and rabbit produced an initial leukocytosis (largely neutrophilic) which reached its maximum in 3 hours and had returned to a normal level in 8 hours. The leukocytosis was then followed by leukopenia which autopsy marrow studies demonstrated was due to degeneration of the marrow. Localized irradiation produced a

transient and less marked leukocytosis (neutrophilia) again followed by a return to a normal level after 6 hours and a continued fall to a leukopenia (with neutropenia) after 24 hours. These early workers described the initial leukocytosis as due to an emigration of neutrophils into the circulating blood from the bone marrow and body tissues where they had been more or less dormant. They envisaged this emigration as a response to irradiation damage, in the same manner that leukocytes emigrate to a point of infection. This explanation may still have merit, but it would seem rather that the neutrophilic increase is due to an outpouring into the circulating blood by the accelerated activity of the marrow.

Other evidence of the supression of the accelerated maturation of neutrophils as a response to larger doses of irradiation is seen in the liberation into the blood stream of young or immature forms following large doses of localized or moderate doses of whole-body irradiation.[76,63] Here the destructive effects are sufficiently great to exhaust the marrow, and it can no longer respond by repopulating the blood stream with mature neutrophils. As a result, immature forms appear in the circulation.

The irradiation of bone marrow cultures has also brought out the variance of leukopoietic response. Osgood and Bracher[75] irradiated cultures of human marrow with doses of 400 r. There was a transient increase in the myeloid elements which persisted for an average of 2 1/2 days and was then followed by a fall in their levels to less than 1/2 normal as the production of neutrophils fell off due to destructive action of the irradiation. There was demonstrated, however, an initial increased production of granulocytes, or probably more correctly, an acceleration in the maturation of granulocytes as the first response.

The so-called "stimulative" effect of irradiation is, however, short-lived for neutrophils and most experimental work has utilized doses of an order of magnitude which render these effects minimal as compared with the repressive effect upon the bone marrow. Practically all the studies after 1905 have confirmed Henicke's[32] original observation that irradiation exerts its neutropenic action through the depression of bone marrow activity. This original observation described noticeable degenerative effects on the marrow 3 1/2 hours after massive whole-body irradiation in animals with marked leukopenia noticeable 48 hours after exposure. There was a greater reduction in the lymphocytes than in the neutrophils. The bone marrow after 2 to 3 days showed marked aplasia. The origin of the neutropenia was ascribed to the effect of the irradiation on the production of the cells in the marrow, rather than a direct effect upon the circulating granulocytes of the blood. All later work tends to confirm this original observation, only to add certain quantitative relations of dose to effect.

Lacassagne and Lavedan[50] studied bone marrow effects in rabbits at varying periods after massive whole-body irradiation. Factors: t.s.d. 50 cm, spark gap distance 40 cm, 3 ma, 6 mm Al, time of exposure up to 4 hours. With these factors of exposure to whole-body irradiation in rabbits, the sequence of events in the circulating blood appeared as:

Immediately after irradiation: A leukopenia developed within 2 hours after the completion of treatment, the neutrophilic loss being less marked than the lymphocytic. The original leukopenia may be a loss of 80 per cent of the pre-irradiation level.

24 hours: Total white blood count 12,000 with 95 per cent neutrophils, 2 per cent lymphocytes, and 3 per cent monocytes.

48 hours: Total white blood count 1800 with 80 per cent neutrophils, 10 per cent lymphocytes, and 10 per cent monocytes.

72 hours: Total white blood count 500 with 50 per cent neutrophils, 10 per cent lymphocytes, and 40 per cent monocytes .

96 hours: Total white blood count 1500 with 40 per cent neutrophils, 20 per cent lymphocytes, and 40 per cent monocytes.

8 days: Return to normal level, 10,000 white blood cells, with 40 per cent neutrophils, 50 per cent lymphocytes, and 10 per cent monocytes.

Bone marrow studies at times corresponding with the circulating blood levels showed:

24 hours: Disintegration particularly of the myeloblasts and myelocytes.

48 to 72 hours: Gross depopulation of the cellular elements of the marrow, leaving largely a connective tissue framework.

96 hours: Beginning repair and reappearance of the cellular elements which increase in amount and are well advanced by the 8th day.

These studies of Lacassagne and Lavedan confirmed those of Henicke, and, in addition, showed that the fall in the actual number of circulating neutrophils is not as marked as that for the lymphocytes; that the picture in the circulating blood and the corresponding state of the marrow correspond in time; and that regeneration of the marrow (for the doses used) could be attained after a severe leukopenia (1500) and neutropenia (600) within a period of about 8 weeks.

Henshaw and Nettleship[34] have recently reported their observations in mice following whole-body radiation of 50 r. Blood counts and tissue sections were made at intervals from 1 to 96 hours postirradiation. They found the initial leukopenia (lymphopenia) followed by the secondary leukocytosis (neutrophilia). The interesting phase of their observations, however, concerns the evident destructive effects seen in the intestinal tract, bone marrow, spleen,and testicle within 1 hour after irradiation. In all tissues examined they found a shift to an acidophilic cytoplasm as evidenced by staining reaction. In the intestine there was present an intense cloudy swelling of the lining cells within 1 hour after irradiation. At the same time they found small areas of necrosis in the germinal centers of lymph nodes and spleen. There was already noticeable destructive effect in the seminal tubules. The bone marrow showed evidence of damage with the formation of giant cells. In no instance did they find evidence of so-called stimulation. Repair in all organs examined was rapid and had been completed within 96 hours. It is conceivable, as these workers pointed out, that had examination been done only at intervals later than 24 hours, when reparative processes were already begun, the interpretation of alterations seen might have been one of a "stimulative effect" rather than as repair of the damaging effects of radiation which were evident within 1 hour after irradiation.

The transient leukocytosis may be the response of the marrow to the initial radiation by an acceleration in the transition from immature to mature neutrophils. There is, however, the damaging effect upon the parent cells which accounts for the neutropenia which follows when radiation is prolonged or intense.

Piney and Mayneord[76] have given data on varying doses (measured in roentgens) of the whole-body irradiation in rabbits in which interesting effects were demonstrated upon the bone marrow as seen in the circulating blood. Factors of the radiation were: 90 kvp, 2.9 ma, H.V.L. 0.295 mm Al, t.s.d. 24.5 cm, 19 r per minute. In the first hours after irradiation with single massive doses of 3000 to 7900 r, large numbers of immature neutrophils appeared in the blood. Five hours after beginning an exposure of 5090 r (and 1/2 hour after completion of the exposure) there was a beginning leukocytosis (neutrophilic) which reached its maximum by the 17th hour when the number of circulating leukocytes was 32,000. The composition of this neutrophilia was largely one of young and immature forms released from the bone marrow. With these massive single doses, the relative neutrophilia (with lymphocytic leukopenia) persisted, although less intensely, until death.

Smaller single doses of the order of 500 r likewise produced an immediate leukopenia with lymphopenia which later persisted for about 6 weeks after irradiation and then gradually returned to normal (Figure 3). The persistent neutrophilia associated with the secondary leukocytosis persisted for about 3 weeks before returning to a normal level.

When these varying doses were divided over a period of days (7 to 23 days) rather than administered as a single exposure, it appeared that the damage was more intense and more prolonged

than when the same amount was given as a single dose (Figure 2). In either case, the daily exposure was of the order of 22 to 1130 r per day. The lesser of these daily exposures over a period of 23 days leaves no margin for recovery from day to day, and the larger dose of 1130 r each day for 7 days amounts to more than a lethal dose repeated daily for 7 days. In other words, had 1130 r been given only once, the bone marrow effect would still have been so overwhelming that recovery was not to be expected. Repetition of this dose each day seemed to have an inhibitory effect on any slight recuperation which may have been possible after the first dose.

At first glance these conclusions of Piney and Mayneord would seem to be at variance with other radiobiologic effects, as, for example, the production of skin erythema. When we consider, however, that in each case the daily dose (whether 22 or 1030 r) was above the threshold effect for the hemopoietic tissues, the deduction is not at variance. To carry the question somewhat further — when the dose is below the threshold effect (0.1 r per day) it can apparently be given indefinitely without detectable effect on the blood forming organs.

The effect of irradiation on the circulating neutrophils is one which thus far has eluded detection if it exists. Various procedures have been devised to detect possible effects upon the circulating neutrophils but none have been conclusive. Jolly and Lacassagne[43] irradiated blood in vitro which was kept under physiologic conditions, but could find no variation from the controls in the doses up to 2800 r. This study was carried further by Lacassagne and Gricouroff[51] by using the motility of the leukocytes under physiologic conditions in vitro as an index of viability. In doses up to 3500 r there was no variation in motility of the neutrophils as compared with controls.

Jansson[41] used a similar technique to study the effect of radiation on the motility of leukocytes. These cells lived normally 48 hours and maintained motility under the conditions of his experiment. The slightest degree of damage was evidenced by slowing of ameboid movements. By varying the time of radiation exposure, he found that 75 minutes exposure produced a marked loss of motility. More severe degrees of injury were evidenced by loss of motion with persistent intracellular movements of granules, loss of boundaries, grouping of granules into large masses, and disintegration in some cells, with recovery in others.

Lacassagne and Gricouroff[51] also attempted to study the phantom "leukotoxin" to which some workers had ascribed the effect of radiation leukopenia. Two rabbits received whole-body irradiation with doses of 1500 to 2000 r, respectively. Total and differential blood counts were done 4, 24, 48, and 72 hours after irradiation. The animals developed the anticipated leukopenia and subsequently died. At the above intervals blood was drawn from the irradiated animals and from controls and mixtures of plasma and leukocytes were made in the following manner:

Normal plasma and normal leukocytes.

Normal plasma and irradiated leukocytes.

Irradiated plasma and normal leukocytes.

Irradiated plasma and irradiated leukocytes.

In none of these mixtures maintained under physiologic conditions in vitro were there noticeable variations from the normal in the survival or motibility of the cells.

It has been suggested (Jacobson) that neutrophils may be damaged in the circulating blood and selectively removed from the circulation by the reticulo-endotheliol system. This assumes that the damage is not one which we can detect by known methods but not beyond the detection and selective withdrawal from the circulation by the spleen as occurs in the removal of the abnormal red blood cells in congenital hemolytic icterus.

LIFE CYCLE OF THE ERYTHROCYTE AFTER IRRADIATION

The red cell series is no more resistant to alteration that the white cell series as a result of irradiation, but the nature of the life cycle is such that the effects are not as well seen.

In the bone marrow, Henicke[32] described a greater latent period for the appearance of the effects of irradiation than existed for the lymph follicles. He first noted breakup of the nuclei of the cells of the bone marrow in about 48 hours after "prolonged" exposure to x ray. In the 48 to 72-hour period there is a gradual disappearance of bone marrow elements, except for a few cells of each type.

He noted recovery beginning in the marrow about the 6th to 10th day, depending upon the size of the dose.

Tsuzuki[93] found no changes visible in the bone marrow of a rabbit femur until about 300 r had been given to the skin on the back. He used x ray of the following factors: 170 kvp, 2 ma, 0.5 mm Cu, t.s.d. 40 cm, SED, estimated at 750 r by Dr. Failla. This was estimated as a local dose in the femur of about 85 r. He then saw degeneration of the nuclei of the younger cell forms. After 24 hours, he saw degenerative changes in the mononuclear, or blast cell forms, both erythro- and myeloblasts. After 48 hours, an increase in pigments and of the mitotic figures of the erythroblasts was seen. No change was noted from this picture after 72 to 96 hours.

After a dose of about 450 r to the skin of the back, or an estimated femur tissue dose of 130 r, he noted the following changes:

<u>Immediately after irradiation</u> there was no change in the erythroblastic series.

<u>After 12 hours</u> an increase in the mitotic figures of the erythroblasts and of the pigment cells was seen.

<u>After 36 hours</u> the same picture as after 12 hours was present.

Immediately after a dose of about 600 r to the skin of the back, or an estimated femur dose of about 180 r, Tsuzuki noted degeneration or destruction of the mononuclear cells and of erythroblasts. After 12 hours pigment cells were observed. In 96 hours, no degenerative forms were seen; mitotic figures of erythroblasts were seen.

Lacassagne and Lavedan[50] described daily examinations of marrow following whole-body irradiation in the rabbit. They found that the erythropoietic elements were affected in the same degree as the leukopoietic elements. By the 3rd day after a single exposure (non-necrotizing dose) the erythropoietic cells were for the most part gone. Repair began on the 4th day and progressed more rapidly than the white cell regeneration. Because of the more rapid regeneration, the longer life span, and the fact that under normal conditions the red blood cells do not migrate from the blood stream, there is not the early appearance of anemia as there is of leukopenia. They suggested that radiation may hasten maturation of the more mature cells of the erythroblastic series, and thus account for the slight elevation of the red blood cell level which may be seen early after exposure.

The Effect of Radiation on the Circulating Red Blood Cells

Most of the work done with red blood cells in vitro would indicate that doses much higher than those used therapeutically or experimentally are needed to produce changes in these cells.

Fricke and Peterson[27] found that 56,000 r were necessary to change a 50 per cent aqueous solution of hemoglobin to methemoglobin. Shouse, Warren, and Whipple[88] found that irradiation in vitro up to 400 milliampere hours had no effect on the ability of red blood cells to resist hypotonic salt solutions. Ting and Zirkle[92] found that exposures of 33,000 r were necessary to render cell membranes permeable to magnesium ions but not to sodium and potassium ions.

Holthusen[37] demonstrated that 50 SED were necessary to produce in vitro hemolysis of erythrocytes. Tsuzuki,[93] however, has observed marked erythrophagous phenomena in the sinuses of the lymph glands before the local destruction of the lymphocytes has occurred. He feels that these erythrocytes are being destroyed, and that the above phenomena indicate that the mature red blood cells are, in some way, damaged relatively early and are altered so that they are readily phagocytized. Henicke,[32] Clarkson et al[16] have observed increased pigmentation in the spleen following irradiation in experimental animals.

Lacassagne and Lavedan[50] working with rabbits found little change in the red blood cell levels. At times a slight rise was observed followed by a mild anemia.

Clarkson, Mayneord, and Parsons [16] using rats observed a marked early anemia after whole-body irradiation with around 500 r. They used x ray with these factors: 150 kv, 4 ma, 3 mm Cu filter, t.s.d. 31.5 cm 30 r/min. With these conditions a MLD was found to be of the order of 350 r in one exposure. The red blood cell count dropped precipitately, as much as 2 or 3 million, in the day or so preceding death. The hemoglobin levels did not drop so rapidly as the red blood cell levels, giving a color index of greater than one. A marked lymphopenia and thrombocytopenia were found also at first; marked atrophy of the lymphoid tissue was noted. Nothing is said of the bone marrow. Bleeding did not seem to be a contributory factor in the animals that died. No other workers have reported these early changes after so little irradiation in other mammals.

Ducuing et al[22] used whole-body radiation in dogs. 500 r in a single exposure produced an aplastic marrow after 17 days (Figure 7). It would seem that there had been complete destruction of bone marrow elements. The red blood cell level fell from 6.8 to 5.1 million in 17 days (see figures). Some of this loss is due to bleeding as a result of the loss of platelets.

The effect produced by 775 r given as 25 r daily exposures over a period of 82 days is best seen in the accompanying Figure 5. This shows a marked anemia at its lowest point 12 days after cessation of irradiation with thereafter a gradual recuperation to within normal limits 60 days after treatment was stopped.

Lacassagne and Lavedan[49] irradiated rabbits 2 days before term, then studied the red blood cell levels of the young. They found a reduction on the 10th day in the red blood cells of 3.0 million (5.0 to 2.0) as compared with the normal.

Wright and Bulman[101] using x ray of the following factors: 20 cm spark gap, filter 1 mm Al, t.s.d. 57 cm, exposure time 30 minutes, lethal dose 60 to 75 minutes, found a drop of the red blood count on the 9th day postirradiation in cats. This drop lasted for about a week and was followed by a slight rise above the pre-irradiation levels.

For the small, single doses, Lavedan and Lacassagne[50] found that the bone marrow, though hit as severely as the lymph follicles, was well on the road to a normal picture in 8 days after the exposure.

Ducuing[22] has shown with the dog that received 31 exposures of 25 r each in 82 days that the marrow is affected by such doses and that return to normal function, judging by peripheral red blood cell levels, occurred about 60 days after the termination of treatment.

In humans, as has been shown by Mottram,[64,65] Martland,[58] Weil and Lacassagne,[94] the bone marrow does lose its powers of recovery and an aplastic anemia develops. Two types of reaction are commonly described, hypoplastic and hyperplastic, the former usually assigned to external, the latter to internal irradiation. The type of exposure producing an aplastic anemia is of an entirely different nature than any that has been experimentally used so far. It consists of daily exposures to small doses of whole-body irradiation of the order of perhaps .5 to 1+ r per day, of irradiation for periods of years, or of exposure to the rays of absorbed radioactive materials. In the case of external irradiation, Mottram[64,65] has described the changes very well. Two workers at a radium institute in England were noted to have had a mild anemia with a high color index for a number of years.

They had been working with radium for a period of 10 years or so, in poor working conditions. Within a period of 2 years, they both developed weakness and malaise which rapidly passed into inability to work and, within a short time, death. The blood picture during the acute, terminal illness was one of failure of production of all cellular elements by the bone marrow. The red blood cells, platelets, and leukocytes were all found in greatly reduced numbers.

Others, Larkins,[52] Faber,[24] and Amundsen[2] have described similar cases. In each case autopsied, the bone marrow was seen to be almost devoid of cellular elements capable of producing blood cells. All cases have in common a long history of exposure to radiation. No ill effects were noted as a rule until years after such work commenced.

Martland[58] has reported on the effects of internal irradiation found in a group of workers from a plant in which dials were painted with a mixture of radium, mesothorium, and radiothorium sulphate. The mode of absorption was mainly through ingestion, resulting from the habit of pointing the radium-dipped brushes with the lips. A secondary route of exposure was inhalation of the radioactive gases. The irradiation was about 92 per cent alpha particles, 8 per cent beta and gamma radiation. The radium was shown to be deposited in the skeletal system almost exclusively, where the bone marrow was constantly and heavily bombarded. The number of persons exposed was about 800, but the known cases developed only in the group that had worked as dial painters for a year or more.

Martland was able to find 18 persons who had died of proved or suspected radium poisoning, and 30 persons alive suffering from symptoms consistent with that diagnosis. In those cases autopsied and studied, the amount of radioactive material in the skeleton was estimated to vary from 10 to 180 micrograms. Rawjewsky[78] estimated a MLD to be of the order of 1 microgram.

Martland found evidences of aplastic anemia in 12 of the 18 cases who had died. Seven of these had a regenerative type of aplastic anemia, 5 had a nonregenerative type. The bone marrow in the nonregenerative type was characterized by a very cellular marrow in which there was a predominance of the younger cell forms and a scarcity of the mature red blood cells. Apparently the effect of the radiation was to stimulate proliferation and mitosis in the immature cells and to inhibit the formation of the mature red blood cells. A sequel to this picture was the encroachment on the marrow by fibroblastic tissue, with the disappearance of the hemopoietic cells. This fibrosis at first was patchy, later involved whole bones.

The peripheral blood picture is very similar to that produced by external irradiation. The red blood count may be less than 1 million with hemoglobin as low as 20 per cent. Anisocytosis and polychromatophilia are frequently noted. A high color index is a very common finding. In many cases the peripheral blood findings may simulate those of pernicious anemia.

The anemias as a result of internal radiation from ingested radium are due mostly to the ionizing effect of the alpha particle. It is for this reason that such small amounts of radium deposited in the bones is fatal.

It should be remembered, however, that the anemias appearing both in humans and in experimental animals following external radiation are due to inhibition or suppression of the erythropoietic system. Because of the long life of the red blood cell, transient fluctuations in the formation of red blood cells are not evidenced in the peripheral blood. It is only after formation has been impaired or suppressed for some time that anemia develops. The acute anemias, produced experimentally, are, as Hilbert and Linzer[36] showed, of the simple normochromic, normocytic type and are due to sudden stoppage of formation. The anemias produced by long-continued daily exposure to very small amounts of external irradiation, such as is found in humans, are associated with abnormalities of regeneration, and one sees abnormally large or small cells and polychromatophilia. The color index is elevated above unity; not infrequently, immature nucleated red blood cells are seen in the peripheral blood stream. Indeed, many of the idiopathic refractory (or aplastic) anemias and even those due to known causes other than irradiation may have a hyperchromic macrocytic anemia. The

bone marrow in this group is often hyperplastic, similar to the bone marrow of some proven cases of radium poisoning.

EFFECT OF IRRADIATION ON PLATELETS

The effect of irradiation on the megakaryocyte and on the peripheral platelet count is a marked one and of considerable importance in the production of acute irradiation death. The marked drop seen after heavy irradiation is a constant finding from species to species, and if severe and prolonged, leads to peripheral bleeding and death.

"With moderate doses," Lacassagne and Lavedan[50] found a reduction of from 300,000 to 30,000 in the platelet count in rabbits on the third day. By the fourth day, the count had again begun to rise. Those findings agreed with their studies of bone marrow in which they found a diminution to absence of the megakaryocytes by the 3rd day, followed by their beginning reappearance on the 4th day.

The same investigators[49] irradiated pregnant rabbits two days before delivery and then followed the blood count in the offspring. They found a thrombocytopenia present throughout the life of the offspring, which was 10 days.

Shouse, Warren, and Whipple[88] gave to dogs 1050 ma.m. total-body irradiation (60 cm t.s.d., 20 kv, 35 ma, filter 1 mm Al). On the 5th day following irradiation they noted a diminution in the number of platelets and these disappeared on the 6th or 7th day; death occurred on the 9th day. All body organs showed extensive and generalized capillary hemorrhage. Studies of the bone marrow showed absence of megakaryocytes. The authors felt that death was due to absence of platelets in the blood.

Ducuing et al[21,22] working with dogs observed marked changes in the platelet count. One dog, after 500 r in one exposure, dropped gradually to a zero platelet count. In 15 days widespread hemorrhage was seen, and the bone marrow was without signs of activity (Figure 7). Another dog received 700 r divided into 3 equal daily doses. The platelet count dropped to zero in 12 days. He was killed 5 days after the platelet count was at zero. Extensive hemorrhage was noted in the skin, mucous membrane, bowel, and lung. The bone marrow showed a few megakaryocytes. A third dog received 775 r in 31 exposures of 25 r each in 82 days (Figure 5). The platelet count began at 400,000, dropped to 10,000 three weeks after the exposures were finished, or 103 days after the irradiation was begun. Recovery was gradual and extended over a period of several months. The dog at no time showed spontaneous hemorrhage. Prolonged bleeding from trauma was noted during the period of severe depression of the platelet count.

Lavedan,[53] when studying patients receiving local x-ray treatment for carcinoma of the cervix, did not find any diminution in the platelet count, but did observe a prolongation in the bleeding time during the first few days.

Ducuing and others[21] report a case of a man with leukemia who received "through error in prescription" 50 r body irradiation every other day for a total of 600 r. He showed spontaneous hemorrhage at the end of his treatment and was kept alive only after strenuous supportative measures.

RADIATION DEATH

In the acute experiments in animals by Ducuing et al[21,22] and Shouse, Warren, and Whipple,[88] death was from widespread purpura resulting from the abrupt fall of the platelet level of the blood. In this case death is essentially one of hemorrhage.

In the more chronic exposures which lead to death, the actual cause or causes of death are more obscure. Henshaw has produced death in mice in 7 to 9 months with daily exposures of 5 r per day, 5 days per week. The control mice live out their normal span of 20 to 24 months. Autopsy does not show that purpuric hemorrhage is present. Actually the mice appear to be in a fairly good general

condition until shortly before death. Autopsy findings have thus far failed to show a specific cause of death. The gonads are atrophic and the lymphoid organs and bone marrow show evidence of marked aplasia, with anemia. With long-continued exposure, death must so far be ascribed to the anemia resulting from erythropoietic damage. In humans, this exhaustion apparently takes years to develop when exposure is of a chronic low intensity. When the anemia does appear, it seems to progress with considerable rapidity. It is characterized by a high color index and many abnormal erythrocytes in the peripheral blood. There is commonly a marked leukopenia and a low platelet level. In occasional instances one sees a hyperplastic marrow which is filled with immature cells, which apparently cannot, or have lost the ability to reproduce mature functional cells.

To date, no valid deductions can be made relative to the incidence of leukemia after long-continued exposure to small doses of radiation. Evans and Roberts,[23] in examining the reported cases up to 1928, could not verify unconditionally that there was a correlation. The report of such cases as those of Weil and Lacassagne[94] in which two workers developed blood dyscrasias (one leukemia, the other aplastic anemia) while working together and receiving similar exposure and dying within a time relatively close together, and certain animal experiments[68,69,71,81,82,83] which have produced an extreme degree of lymphocytosis, would lead one to think that the hazard of leukemic production is possible.

Radiation Injury from Internal Irradiation

1) Experimental: Jansen and Schultzer[42] describe one of the few experiments with this type of irradiation. They kept rats in an atmosphere of 2000 Mache units of radon per liter of air for a period of 5 weeks. Blood counts showed an initial leukocytosis, then an irregular fall which in but 1 case out of 4 fell below the initial count. The percentage of mononuclear cells was not markedly altered. It was noted that the coagulation time was markedly shortened. The red blood cells were not affected. If suckling rats were used, the 5 weeks exposure produced a definite leukopenia with a relative and absolute lymphopenia showing comparable results with those that Lacassagne had previously obtained with external irradiation of rabbits in utero.

Microscopically, there were many hemorrhages in the lungs. Lymph follicles in the spleen showed numerous pyknotic lymphocytes; the germinal centers were atrophic. In addition, the dose was sufficient to produce failure of development of the testes.

If rats were kept in an atmosphere of 500 Mache units, the weight curve was not altered from normal. Unfortunately, blood counts were not taken.

Schultzer,[87] in an attempt to show that the above effects were due not to the soft irradiation produced by the radon but were due rather to the gamma irradiation, placed paraffin capsules containing radium bromide within the peritoneal cavity of numerous rats. Even with the highest dose, using .04 mg of radium bromide, the weight curves of the rats were not affected for a period of 9 weeks. Unfortunately, no blood counts were taken.

2) Clinical: Martland's work[58] on the effects of ingested radium was the first to call attention to this hazard. Up to 1931, a period of about 10 years following the onset of the exposure, 18 deaths had occurred in which radiation poisoning was proved or suspected. There were 30 persons alive suffering from conditions which had symptoms compatible with radium poisoning. Ten of those who died were found to have an anemia as a primary or complementary diagnosis. Histology of the bone marrow showed three distinct phases. The first was a so-called hyperplastic phase. This phase might exist for years and was, he felt, produced by a failure of the cells of the bone marrow to mature. The picture is characterized by the large numbers of primitive cells seen and by a low number of mature forms. The second type of picture is seen when an invasion of very cellular fibrous tissue begins. This invasion is patchy and may coexist with the above picture. The final picture is a loss of the cellularity in the connective tissue, a moderate amount of bone absorption with deforming lesions of the bone resulting. All three of these pictures may exist in the same marrow.

Blood pictures associated with these changes are those of profound anemia, often of a million or less, with an increase in the color index, many abnormal red forms, and an occasional immature form in the circulating blood. The white blood count is frequently between 1000 and 2000 cells per cubic millimeter. There is a relative increase in the lymphocytes. Platelets are frequently diminished and the icterus index is low.

Schlundt, McGavock, and Brown[86] describe the effects produced in men working in an atmosphere of thorium and thoron. An electroscope to determine minute amounts of thoron in air and expired air is described. With the thoron content in the air of an average of about 4.0×10^{-4} curies per cubic meter, definite blood changes were found. In the figures given, the average white count is about 4000 with a differential count of about 55 per cent neutrophils, 37 per cent lymphocytes, and 8 per cent monocytes and an occasional reversal of the lymphocyte-neutrophil ratio.

McClelland[70] gives a series of tables pertaining to amounts of radon in the expired air with blood counts from which it was possible to compile a graph in one person showing a correlation between the level of the white blood count and the amount of radon in the expired air. On three occasions this person had reversal of the lymphocyte-neutrophil ratio following quite high exposures to gas. After his work was discontinued, the lymphocyte-neutrophil ratio returned normal.

The effects of internal irradiation can be found to be of the same order as those produced by external irradiation. The fact that alpha irradiation is present in cases in which ingestion or inhalation has occurred makes smaller amounts of material necessary to produce blood changes than is the case for external irradiation. However, in general, the effects upon the white blood counts are quite comparable. The anemia, however, is more rapidly produced and more severe.

SUMMARY

Lymphocytes

The formation of lymphocytes is markedly altered after irradiation with x or gamma rays. The effect can first be seen after relatively small exposures. Destruction of lymphocytes in the exposed spleen or intestine have been noted after radium exposure calculated to be only approximately 0.25 r.[33] Henshaw has found alteration in lymphocytes after 50 r body irradiation in mice. Tsuzuki found destructive changes in the lymphocytes in lymph nodes after 75 to 150 r body irradiation in rabbits. The degree of destructive changes is correlated with the size of the dose through a considerable range. After exposures of the order of 800 to 1000 r are exceeded, the effect on the formation of lymphocytes is maximal.

Beginning return of the lymphocyte formation, after doses of the order of 200 to 300 r, is usually noted around 6 days after the exposure was given. The time interval between irradiation and beginning recovery is directly proportional to the magnitude of the exposure, if single exposures only are considered, for doses above about 100 r and less than 800 to 1000 r. Within limits, fractionation and protraction of a given dose can prolong the period of decrease in lymphocyte formation.

The numbers of circulating lymphocytes are markedly altered by whole-body irradiation. The variations can be correlated with the picture in the reticulo-endothelial system, and are a reflection of the pathologic process in these organs. After single exposures to 50 r or more total-body irradiation, a marked drop has been noted in the number of circulating lymphocytes. This decrease usually begins within one hour after the irradiation was begun. The drop corresponds with the onset of destructive changes within the reticulo-endothelial system. By 4 to 8 days the count will be at its lowest point and may be as low as 400 lymphocytes after a single exposure of 200 to 300 r. Throughout this period evidence of alteration in lymphatic tissues continues. From the 4th day onward, the lymphocyte count begins to increase. This increase is concomitant with the beginning of recovery in the lymphatic tissues which consists of repopulation of lymphatic tissues with lymphocytes.

Evidence of destruction of circulating lymphocytes in vivo has not been presented. In vitro experiments indicate that around 2000 r are necessary to produce loss of motility in lymphocytes obtained from the circulating blood.

It has been shown that irradiation of single large fields with moderately penetrating x ray can also produce a lymphopenia. The reduction in the lymphocytes first appears 2 to 3 hours after irradiation begins, reaches its maximum 3 to 5 hours after irradiation, and has returned to essentially normal limits within 24 hours. A second exposure 24 hours after the first gives a repetition of the process, but to a greater degree. After 10,000 to 12,000 r have been given in 3 weeks to the pelvis, the reduction in the number of lymphocytes may amount to 50 per cent of the original number. Recovery commonly occurs in a 4 to 5-week period. In some instances the depression of the lymphocyte blood count is sufficient to necessitate interrupting treatment.

Polymorphonuclear Leukocytes

One of the first effects of total-body irradiation upon the myeloid series after a single exposure of 200 to 300 r is acceleration of the process of maturation of the immature cells. The effect is transient and is lost in 12 to 24 hours. It is followed by degenerative changes, most marked in the myeloblasts and myelocytes.

Twenty-four hours later, the decrease in the numbers of polymorphonuclear elements is striking. Repair may begin as early as 4 days. The greater the dose, the longer will repair be delayed. Doses greater than 300 r enhance the severity of the degenerative changes but do not alter their fundamental character. (Henshaw has shown that changes may be seen after 50 r whole-body irradiation in mice.)

The changes in the number of circulating polymorphonuclear leukocytes alter with the changes in the bone marrow. Coincident with the hastening of the maturation process, and usually appearing about 5 to 7 hours after the onset of irradiation, there is a leukocytosis produced by an increase in the numbers of the circulating polymorphonuclear leukocytes. Many of these cells are immature. This leukocytosis is transient and usually disappears within 24 hours after its onset. It is followed by a leukopenia, the duration and severity of which is correlated with the amount of destruction which has been produced in the bone marrow. This, in turn, is a function of the amount of radiation given. Within limits, the changes after fractionation and protraction of whole-body irradiation tend to become more severe as the dose is divided.

Within the area irradiated, the effects of irradiating a large single field or of multiple fields are the same as those described above. Some evidence has been presented supporting the thesis that the unirradiated bone marrow will undergo compensatory hyperplasia in an attempt to maintain the normal level of the circulating blood. However, evidence has also been presented indicating that this hyperplasic reaction ultimately fails to compensate for the bone marrow damage in the irradiated part. Whether an irreparable leukopenia or anemia resulting from long-continued local irradiation is due to the final exhaustion of the nonirradiated marrow or is due to some other as yet unexplained mechanism remains an unsolved question.

Monocytes

No very clear picture has been formed as to the response of the monocytes to irradiation. There does not seem to be any characteristic response. There is, however, a feeling among those who observe the changes in radiological personnel that a monocytosis indicates that a considerable degree of damage has occurred. Goodfellow states that persons showing a persistent, absolute monocytosis, especially if the lymphocyte-monocyte ratio is reversed, should be removed from further exposure.

Red Blood Cells

It seems well-established that the formation of the red cell series in the bone marrow is altered by exposures to radiation, and that the doses need be no larger than those necessary to produce changes in the myeloid series.

Few workers have noted any change in the red blood cell counts after single exposures to radiation, even of large amounts. The explanation lies in the long life of the circulating red blood cell. Recovery of erythrocyte formation after single exposures of 300 to 500 r is within 14 to 20 days, and the marrow is functioning again before any change in the number of circulating erythrocytes has developed. When whole-body irradiation is given, the parent cells of the erythrocytes are affected throughout the bone marrow. After local irradiation the same changes are produced in the marrow irradiated.

After whole-body irradiation with doses of 120 to 300 r, some observers have described an early, transient increase in the reticulocyte count. Others do not find it. The effect has not been looked for often enough to be sure of its presence or absence.

Within 24 hours after doses of 300 to 500 r total-body irradiation, the cells of the erythroblastic series show signs of degeneration — nuclear karyorrhexis and pyknosis. Within 98 hours after doses of the above magnitude, the number of the cells visible on sections of bone marrow has markedly diminished. With the higher doses, virtual depopulation of the bone marrow may occur. Recovery in these elements, as in the myeloid and lymphocytic elements, varies directly with the magnitude of the total dose. Also, within limits, fractionation and protraction of a relatively large dose prolongs the effect on the erythroblastic series.

Platelets

Platelets are formed in the bone marrow. Their progenitors, the megakaryocytes, react in the same fashion to irradiation as do the cells of the erythrocytic and myeloid series. The platelet level in the circulating blood varies with the degree of damage to the megakaryocytes. Their number in the blood varies rapidly, probably due to their short life, e.g., if formation is altered, their numbers will quickly change. If the number of platelets is depressed below 20,000 to 40,000, bleeding into the tissues and from mucous surfaces ensues. Acute irradiation death (5 to 20 days) after single massive exposure of whole-body irradiation is preceded by diminished to absent platelets and by generalized hemorrhage. This deficiency in platelets is probably the greatest single factor in this type of irradiation death.

After chronic, long-continued exposure of the whole body to radiation, as in radiation workers, death is frequently preceded by a deficiency in platelets and by capillary bleeding. However, here the platelet count will be found to be within relatively normal limits until a short time before death.

CONCLUSIONS

This summary was undertaken in an attempt to determine which elements or element of the blood would give the most sensitive index of irradiation damage. The information at hand is incomplete and in many ways unsatisfactory both because of uncertainty as to the details of the exposure and dose, and because most of the experiments described above are of an acute nature. Our problem is a quite different one in at least two important, and perhaps fundamental, respects. The exposure is far less than that used in any, save a few, of the experiments we have discussed, and exposure is prolonged over months and years, instead of being given in a few days or weeks. Secondly, in most of the examples cited above, the radiation was either x or gamma rays, and in our case there is the possibility of exposure to alpha, beta, and gamma radiation of varying energies, as well as both slow and fast neutrons.

It should be emphasized again that the problem here is one of chronic exposure, or of chronic plus occasional acute exposure. This type of radiation exposure has not been studied experimentally to any extent. The information obtained from humans exposed to radiation is scant and unsatisfactory because little information as to the amount of exposure is given.

Another point which deserves mention again briefly is the possibility of alteration in the blood reaction due to the combination of the various types of irradiation.

The tolerance dose which is being used will most certainly have to be revised for some of the nuclear elements. Tolerance dose for fast neutrons, for example, has already been changed to 1/10 the value for gamma radiation. It is by no means certain that the effects produced by the varying types of exposure will be comparable, or that exposure to one form affects the tolerance for other forms of radiation.

Finally, we should like to emphasize that, regardless of the conclusions drawn, the blood studies done here should be as complete as is feasible with the equipment and personnel available. This holds also for the extensions of this project to other geographical areas.

1) The lymphocyte and its predecessors are the most sensitive cells in the blood system. This seems well-established from both the acute experiments and from the observations made in humans exposed to irradiation for long periods of time. The characteristic response is a diminution in the numbers of circulating cells. The degree of diminution depends upon the exposure. Another possible effect of radiation upon the lymphocytes is an increase in their number. This response apparently needs very soft irradiation to call it forth. Russ, Nakahara, and others have demonstrated its occurrence both after single and repeated exposures. It is possible that we may see this type of response in some personnel exposed to beta or low energy gamma rays. The first-mentioned effect, however, is far more common.

2) The polymorphonuclear leukocytes and their predecessors seem to be somewhat less sensitive to radiation than are the lymphocytes. Any alteration in their numbers would almost certainly be accompanied, or preceded by, changes in the circulating lymphocytes. It may eventually be possible to obtain an approximation of the exposure by the interplay of these two blood elements. The earliest change which has been found in the neutrophilic series has been an increase in the rate of maturation of the myeloid series in the bone marrow. It has been shown that exposure of humans to 28 r whole-body irradiation hastens maturation. One can use sternal puncture of biopsy as a means of evaluating the damage done to the hematopoietic system. However, this method is hardly available for routine frequent use. The circulating neutrophils can be seen to vary numerically after irradiation. These changes are now used in evaluating the blood effects of irradiation. We should regard a "shift to the left" in the differential count of the neutrophils as evidence of definite overexposure in absence of other causes.

3) The red blood cells are relatively insensitive to the effects of irradiation except for long-continued exposure. Hence, alterations in red blood cell counts would not be expected to give evidence of overexposure at an early date. If the red blood cell count does begin to drop, it must be regarded, in the absence of other known causes, as strong evidence of a severe degree of bone marrow impairment. Some workers, notably Moldslowsky, have pointed out the possibility that the reticulocyte count is a sensitive index of exposure to radiation. In so far as we know, this evidence does not warrant a definite conclusion. It is possible that the reticulocyte count will assume a more important place in the evaluation of irradiation injury than it now has. Further experimental and clinical evidence must be obtained. Certainly the erythropoietic series in the bone marrow has the same order of sensitivity as has the myeloid series, and seems to go through the same hastening of the process of maturation after irradiation.

4) Platelets have been shown to be a fairly sensitive index of irradiation damage to the blood system in the acute exposure to whole-body irradiation. Bone marrow studies show that the megakaryocytes (probably the predecessor of the platelet) has the same order of sensitivity as has the myeloid series. As yet no one has demonstrated significant alterations in the chronic (radiation personnel) exposures, save in the terminal stages of bone marrow exhaustion. It is also true that few workers have looked for such changes, and that many of the methods of counting platelets are unreliable.

So we feel that platelet counts, with a reliable method, should be done on beginning work with radioactive materials, and subsequently additional platelet counts should be taken if there is any suspicion of acute overexposure.

5) Monocytes are of importance only in evaluating the more extreme degrees of irradiation change. When absolute monocytosis occurs, radiation damage is severe.

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